gradients, or by a combination of the two, so that the buffer **14** moves from the region of low retention factor to the region of high retention factor as indicated by arrow **17**.

[**0026**] In the embodiment of **FIG. 1**, the micelles **12** are negatively charged, and a voltage is applied via power supply **18** to the channel **10** so that the micelles **12** electrophoretically migrate from the region of high retention factor to the region of low retention factor as indicated by arrow **19**. In the region of high retention factor, the analyte moves primarily with the micelles **12**. In the region of low retention factor, the analyte moves primarily with the mobile phase, i.e., buffer **14**. The result is that the analyte moves toward the center from both ends of the channel and is focused at some point F along the retention factor gradient at which the net velocity of the analyte is zero. Although the embodiment of **FIG. 1** has the micelles being negatively charged, one skilled in the art will readily appreciate that the same focusing technique can easily be modified for positively charged micelles.

[**0027**] Although the present micellar gradient focusing bears similarities in its implementation to temperature gradient focusing, micellar gradient focusing focuses analytes via a different mechanism and is functionally unique from temperature gradient focusing. Further, it is important to note that whereas temperature gradient focusing requires a buffer whose ionic strength is a function of temperature, micellar gradient focusing can be accomplished in any buffer capable of supporting a pseudostationary phase.

[**0028**] Micellar gradient focusing requires the production of a spatial gradient in the analyte velocity. For the motion of an analyte in a system such as that illustrated in **FIG. 1**, the total analyte velocity is given by:

$$u_T=(u_B+u_{EP})\cdot[1/(1+k)]+u_{MC}\cdot[k/(1+k)],$$

[**0029**] where u_B is the mobile phase velocity, u_{EP} is the electrophoretic velocity of the analyte through the mobile phase (equal to zero for neutral analytes), u_{MC} is the (total) velocity of the micelles, and k is the retention factor (a factor indicating the relative amount of time the analyte spends in the pseudostationary phase). This description describes methods to produce the required velocity gradient by producing a gradient in the retention factor k.

[**0030**] The retention factor is equal to $k=K\beta$, where K is the distribution coefficient which quantifies the affinity of the analyte for the pseudostationary phase, and β is the phase ratio defined as the ratio of the volume of pseudostationary phase to the volume of mobile phase. The production of a retention factor gradient can then be accomplished either through a gradient in the distribution coefficient or through a gradient in the phase ratio or a combination of the two. There are several different ways that this can be accomplished, some of which are listed below.

[**0031**] A solution of surfactant will form micelles if and only if the concentration of surfactant is greater than the critical micelle concentration (hereinafter "CMC"). For surfactant concentration greater than the CMC, the volume of micellar pseudostationary phase is proportional to the difference between the concentration and the CMC. For most surfactants, the CMC is a function of temperature. Therefore, a gradient in the phase ratio can then be obtained by applying a temperature gradient at a fixed surfactant concentration.

[**0032**] Implementation of this embodiment is described with reference to a microfluidic chip exemplar having a microchannel **20** as shown in **FIG. 2** which is one possible apparatus for controlling the temperature of different parts of the microchannel. The method of creating a temperature gradient is the same as described in U.S. patent application Ser. No. 10/197,331, herein incorporated by reference. However, the apparatus of U.S. patent application Ser. No. 10/197,331 provides for just one of many different ways to manipulate the temperature in a microchannel which are known to one of ordinary skill in the art.

[**0033**] In the embodiment, the buffer is 5 mM carbonate buffer, pH 9.4 in approximately 5% by weight ethanol, 95% water, with a 5 mM concentration of sodium dodecyl sulfate (SDS). For an analyte, the neutral fluorescent dye rhodamine B is added to the solution at a concentration of about 2.5 μM. For this type of SDS solution the CMC is an increasing function of temperature, so that the phase ratio is a decreasing function of temperature.

[**0034**] A microchannel **20** with dimensions 30 μm deep, 50 μm wide, and 2 cm long is used for the separation. The microchannel and fluid reservoirs **27** are initially filled with the uniform sample solution. The temperatures of the cold and hot portions of the apparatus are set to 10° C. and 80° C. using heat sinks such as cooling copper blocks **23***a*, **23***b* covering much of the ends of the microchannel **20**, and one hot zone provided by a heat source such as heated copper block **22**. Thermal contact between the poly(carbonate) and the copper blocks is insured using a thermally conductive adhesive **26**. The copper blocks **22**, **23***a*, **23***b* are arranged so that there was a 1 mm gap **28** between the heated copper block **22** and the cooling copper block **23***a* and a 2 mm gap **29** between heated copper block **22** and the cooling copper block **23***b*.

[**0035**] Microchannel **20** also includes electrodes **25**, **24**, input wells, i.e., buffer reservoirs **27** for the mobile phase buffer, and a narrow hot zone **20***a* near the middle of the microchannel **20**. The heated copper block **22** is heated using a small high-power resistor embedded into the copper and its temperature is regulated using a PID temperature controller (Omega Engineering Inc., Stamford, Conn.). To regulate the temperature of the cold zones, ¼ inch diameter holes are drilled through the cooling copper blocks **23***a*, **23***b* and cold water from a thermostatted bath (Neslab, Portsmouth, N.H.) is passed through them. The amount of solution in the input wells at each end of the channel **20** is adjusted to produce pressure-driven flow so that when a voltage is applied, the micelles would move from cold to hot above the **1** mm gap while the mobile phase would move from hot to cold above the gap.

[**0036**] A voltage of +125 V is applied, and the distribution of rhodamine B dye in the channel is monitored using a fluorescence microscope and CCD camera **32**. A series of the resulting images taken at 10 second intervals from 0 to 50 seconds as is shown in **FIGS. 3***a*-**3***f*, respectively with the rhodamine B identified as B. After 50 seconds, a bright band of focused rhodamine was clearly visible near the hot end (the right side in the figure) of the temperature gradient (**FIG. 3***f*).

[**0037**] The same apparatus as described above can be used to produce the temperature gradient in a capillary. The solution used is similar to that used for the device in a